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SCIENTIFIC NOTE

IVERMECTIN AS A RODENT FEED-THROUGH INSECTICIDE FOR CONTROL OF IMMATURE SAND FLIES (DIPTERA: PSYCHODIDAE)

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ABSTRACT. Ivermectin was evaluated as a potential rodent feed-through for the control of immature stages of *Phlebotomus papatasi*. The survival of sand fly larvae fed feces of Syrian hamsters (*Mesocricetus auratus*) that had been fed a diet containing 0, 2, 6, 10, 20, 60, or 100 ppm ivermectin was measured. Sand fly larvae fed the feces of ivermectin-treated hamsters had significantly reduced survival, with 100% mortality of larvae fed feces of hamsters fed a diet containing 20, 60, and 100 ppm ivermectin. The results of this study suggest that a control strategy using rodent baits containing ivermectin to control phlebotomine sand flies may be possible. Because rodent reservoirs and sand fly vectors of *Leishmania major* live in close association in many parts of the Middle East, the control of transmission of the agent of zoonotic cutaneous leishmaniasis also may be possible.

KEY WORDS Sand fly, larval control, ivermectin, feed-through, leishmaniasis

Phlebotomine sand flies (Diptera: Psychodidae) are important both as biting pests of humans and as the vectors of human pathogens. Most importantly, sand flies are the vectors of the protozoan parasites that cause leishmaniasis. Worldwide, 2 million new cases of leishmaniasis are believed to occur annually, and as many as 12 million people currently may be infected (World Health Organization [WHO] 2006). Throughout North Africa, the Middle East, and Southwest Asia, the sand fly *Phlebotomus papatasi* Scopoli is the vector of *Leishmania major*, which is the causative agent of zoonotic cutaneous leishmaniasis (ZCL).

The reservoir hosts of *L. major* in arid and semiarid Old World foci are burrowing rodents. Sand flies proliferate inside rodent burrows, where the habitat provides high relative humidity and is protected from extreme temperatures. Adult sand flies live in close proximity to sources of blood (from the rodents living within the burrows) and to sugar (from plants that grow near the burrow entrances), whereas the larvae develop within the abundant organic matter inside the burrows. The direct treatment of rodent burrows with insecticides has been largely unsuccessful for controlling sand fly populations. Insecticide applications in and around rodent burrows do not reach the microhabitats of adult or immature sand flies that may be located deep within the burrows (Seyedi-Rashti and Nadim 1973, Karapet'ian et al. 1983). Because leishmaniasis is an uncontrolled and emerging disease that disproportionately affects human populations in developing countries, the development of new, efficacious methods for the control of the vectors of ZCL is needed (Saravia 2004).

In ZCL foci in the Old World, rodent burrows are considered to be the primary habitats for immature *P. papatasi*, and larvae have been observed feeding on the feces of rodents (WHO 1968). Based on this aspect of sand fly ecology, a rodent feed-through method could be a potential means to control sand fly larvae. Proof of concept for this method has been established using 2 chitin synthesis inhibitors (diflubenzuron and novaluron) against larvae of *P. papatasi* (Mascari et al. 2007a, Mascari et al. 2007b). Ivermectin is a macrocyclic lactone that acts as a broad-spectrum endectocide against numerous nematodes and arthropods, and has been shown to have broad insecticidal effects in many feed-through systems, particularly in cattle (Miller et al. 1981). The objective of this study was to assess ivermectin as a rodent feed-through to control sand fly larvae. The development and survival of *P. papatasi* larvae fed feces of Syrian hamsters (*Mesocricetus auratus*) that had been fed a diet containing ivermectin were evaluated.

The sand flies used in these studies were from a laboratory colony of a Turkish strain of *P. papatasi* established at Louisiana State University (Mascari et al. 2007b). Larvae were reared using a lab diet consisting of composted and dried rabbit feces and rabbit chow mixed 1:1 (Young et al. 1981). Adults were provided 20% sucrose solution ad libitum and obtained blood meals from Syrian hamsters. The colony was maintained in

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environmental chambers at 28°C, 90% relative humidity, and 14:10 (L:D) photoperiod.

A total of 24 Syrian hamsters were housed individually in micro-isolator cages as described by Mascari et al. (2007a). The maintenance of the hamsters and all experimental procedures followed Animal Care & Use Protocol No. 05-074, which was approved by the Institutional Animal Care and Use Committee at Louisiana State University, Baton Rouge, LA. Research involving the hamsters was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996).

Two separate feed-through experiments were conducted using different concentrations of ivermectin. Hamster diets were prepared by adding technical ivermectin (Merck & Co., Inc., Whitehouse Station, NJ) to a meal-form laboratory rodent diet (LabDiet® 5001 Rodent Diet; PMI Nutrition International, Brentwood, MO) and thoroughly mixing the diets.

Ivermectin was added to hamster food to obtain diet concentrations of 2, 6, and 10 ppm in the 1st experiment, and 20, 60, and 100 ppm in the 2nd experiment. In each experiment, 3 hamsters were randomly assigned to each of the 3 diet groups containing ivermectin and to a control diet group (0 ppm ivermectin). At 12:00 p.m. each day for 9 days, the hamsters were provided with 15 g of their respective diets. The uneaten portion of the food was collected the following day at 12:00 p.m., and the daily food intake for each hamster was calculated. The daily dosages of ivermectin that were ingested by the hamsters were calculated in milligrams per kilogram body weight. The body weight of the hamsters was measured on the day before the experiment. The feces produced by each hamster were collected daily for 9 days. The feces were dried at room temperature for 7 days and then were stored at −70°C until used.

In each experiment, the body weight and daily food intake of hamsters in the 4 diet groups were compared using repeated-measures analysis of variance (ANOVA) performed with the GLM procedure of SAS (SAS Institute 2001). The Tukey multiple comparison procedure was used to separate significantly different means.

Hamster feces collected during the 1st and 2nd feed-through experiments were assayed separately. The feces voided by hamsters after 9 days of feeding were pooled by hamster diet group, and were crushed using a glass mortar and pestle.

In the 1st experiment, sand fly larvae were fed the feces of hamsters in each hamster diet group (0, 2, 6, and 10 ppm ivermectin). Two control larval diet groups were also included: an untreated control fed standard larval diet to identify potential differences in the survival of larvae fed hamster feces and the standard colony larval diet. A positive control group fed larval diet containing 100 ppm ivermectin was also included. In the 2nd experiment, sand fly larvae were fed feces of hamsters that had been fed a diet containing 0, 20, 60, and 100 ppm ivermectin for 9 days.

Larval bioassays were conducted as described by Mascari et al. (2007a). A 200-mg sample of larval diet (hamster feces or the lab diet) was transferred to the plaster surface of each bioassay vial. Ten 2nd instars (larvae 13 ± 1 days old) were transferred to each bioassay vial and held in an environmental chamber at 28°C, 90% relative humidity, 14:10 (L:D) photoperiod. Six bioassay vials were used for each larval diet group.

The larvae were observed under magnification daily. Larval mortality (defined as the lack of response to prodding with a blunt probe after 15 sec) was recorded, and the larvae were observed for abnormal behavioral and morphological characteristics. Evidence of feeding (the presence of frass in the vials and dark material in the guts of larvae) was also monitored.

Data collected in the bioassays using hamster feces from the 1st and 2nd experiments were analyzed separately. The percent survival of immature sand flies to adult emergence after being fed their respective diets was compared with repeated-measures ANOVA performed with the GLM procedure (SAS Institute 2001). The Tukey multiple comparison procedure was used to separate significantly different means.

In the 1st feed-through experiment, the mean body weight of the 12 Syrian hamsters fed diets containing 0, 2, 6, and 10 ppm ivermectin was 128.2 ± 9.1 g, and the mean body weights of hamsters in these hamster diet groups were not significantly different ($F = 0.87$, $df = 3$, $P = 0.49$). The mean daily food intake of the hamsters was 9.1 ± 1.2, 9.6 ± 1.5, 9.1 ± 1.3, and 8.9 ± 1.4 g for hamsters receiving diets containing 0, 2, 6, and 10 ppm ivermectin, respectively, and was not significantly different ($F = 1.27$, $df = 3$, $P = 0.29$). The estimated mean daily dosages of ivermectin for hamsters were 0.2 ± 0.1, 0.4 ± 0.1, and 0.7 ± 0.1 mg/kg body weight for hamsters receiving 2, 6, and 10 ppm ivermectin, respectively.

In the 2nd feed-through experiment, the mean body weight of the 12 Syrian hamsters that were fed diets containing 0, 20, 60, and 100 ppm ivermectin was 124.2 ± 14.6 g, and the mean body weights of hamsters in the different hamster diet groups were not significantly different ($F = 2.78$, $df = 3$, $P = 0.11$). The mean daily food intake of the hamsters was 7.2 ± 2.6, 7.2 ± 1.5, 5.6 ± 1.1, and 4.6 ± 1.5 g for hamsters receiving diets containing 0, 20, 60, and 100 ppm ivermectin, respectively. The means of daily food intake

Table 1. Mortality of 2nd instars of sand flies fed feces voided by ivermectin-treated or untreated Syrian hamsters, and ivermectin-treated or untreated laboratory larval diet (1:1 rabbit feces–rabbit chow w:v).

Larval diet	Mortality % (mean[1] ± SE)
Experiment 1	
Hamster feces[2]	
0	5.0 ± 5.5
2	85.0 ± 16.4
6	80.0 ± 22.8
10	93.3 ± 12.1
Laboratory diet[3]	
0	8.3 ± 7.5
100	100
Experiment 2	
Hamster feces[2]	
0	5.0 ± 8.4
20	100
60	100
100	100

[1] Six replicates, 10 larvae per replicate.
[2] Concentration (ppm) of ivermectin in hamster diet.
[3] Concentration (ppm) of ivermectin in laboratory diet.

of hamsters fed diets containing 0 and 20 ppm ivermectin were significantly different from the mean daily food intake of hamsters fed diets containing 60 and 100 ppm ivermectin ($F = 10.21$, $df = 3$, $P < 0.01$). The means of daily food intake of hamsters fed diets containing 60 and 100 ppm were 22% and 36% lower, respectively, than the mean daily food intake of the hamsters fed a diet containing 0 ppm ivermectin. The estimated mean daily dosages of ivermectin for hamsters were 1.2 ± 0.3, 2.8 ± 1.0, and 4.2 ± 1.8 mg/kg body weight for hamsters receiving 20, 60, and 100 ppm ivermectin, respectively.

In both the 1st and 2nd experiments, larvae in each of the larval diet groups were observed feeding, and frass was found in each bioassay vial. In the 1st experiment, the mean percent survival was not significantly different between larval groups fed either feces from untreated hamsters or the lab diet ($t = 0.54$, $df = 10$, $P = 0.60$; Table 1). In the bioassay using hamster feces collected in the 1st experiment, the mean percent emergence for the sand fly larvae fed feces from ivermectin-treated hamsters was significantly different from that of larvae fed feces from untreated hamsters ($F = 37.27$, $df = 3$, $P < 0.01$; Table 1). The mean longevity of larvae after they were fed feces of hamsters that had been fed diets containing 2, 6, and 10 ppm ivermectin was 5.3 ± 2.9, 6.2 ± 4.1, and 4.0 ± 3.2 days, respectively. Larvae fed the rabbit feces−rabbit chow diet containing 100 ppm ivermectin all died within 3 days. The larvae that were fed feces voided by ivermectin-treated hamsters and lab diet containing ivermectin became rigid and ceased feeding before they died.

In the larval bioassay using hamster feces collected in the 2nd feed-through experiment, the mean mortality of larvae fed feces from untreated hamsters was 5%. The mortality of larvae that were fed feces from ivermectin-treated hamsters was 100% (Table 1). The mean longevity of larvae after being fed feces of hamsters that had been fed diets containing 20, 60, and 100 ppm ivermectin was 4.5 ± 2.3, 3.5 ± 1.9, and 4.3 ± 2.6 days, respectively. The larvae fed feces from ivermectin-treated hamsters in this bioassay also became rigid and ceased feeding before death.

The sand fly larvae in this study readily fed on hamster feces, including the feces of hamsters that had been fed diets containing ivermectin. Larvae died soon after being fed feces from ivermectin-treated hamsters, typically within 1 wk. These findings are consistent with the findings of Miller et al. (1981) in which horn fly, face fly, house fly, and stable fly larvae died soon after being fed feces from ivermectin-treated cattle.

The quantity of food that was consumed by the hamsters tested in this study was not affected by the incorporation of 2, 6, 10, or 20 ppm ivermectin into their diet. However, hamsters that were fed diets containing 60 and 100 ppm ate significantly less than the control hamsters. The diet concentration of 20 ppm ivermectin did not reduce hamster feeding and was more effective than lower diet concentrations as a feed-through against sand fly larvae. The corresponding mean daily dosage of ivermectin for hamsters fed a diet containing 20 ppm ivermectin (1.16 ± 0.27 mg/kg body weight) was below the LC_{50} observed in orally dosed rats (42.8–52.8 mg/kg body weight), as well as the level at which sublethal effects (such as moderate incoordination) have been observed (4 mg/kg body weight; IPCS 1994).

Previously, diflubenzuron and novaluron were evaluated as rodent feed-through insecticides for immature sand flies, and the feces of hamsters treated with these chitin synthesis inhibitors affected the development of sand fly larvae (Mascari et al. 2007a, Mascari et al. 2007b). Diflubenzuron interrupted the development of larvae during the molt from larva to pupa, and novaluron affected sand flies during larval molts. Sand fly larvae may survive for up to 17 days after ingesting diets containing chitin synthesis inhibitors because these compounds act at specific developmental stages in sand flies. In contrast, ivermectin induces an acute response in insects by enhancing neural and neuromuscular transmission of gamma aminobutyric acid (GABA), which leads to paralysis and death. As expected, sand fly larvae fed feces from ivermectin-treated hamsters died rapidly, and their death was not linked to an event in their development.

Ivermectin has pharmacokinetic properties that make it an appropriate feed additive for the

control of fly larvae that feed on animal feces. Over 90% of orally administered ivermectin is excreted by various mammals (cattle, sheep, pigs, and rodents) unchanged in the feces (Campbell et al. 1983). Ivermectin excreted in animal feces also degrades at a slow rate under field conditions. Sommer and Steffansen (1993) did not observe a reduction in the amount of ivermectin in cow dung that was in a pasture for 45 days, and Madsen et al. (1990) found that dung from ivermectin-treated cattle remained toxic to house fly larvae after 2 months.

The results of this study suggest that ivermectin-treated diets are effective as feed-through for control of sand fly larvae at concentrations that are palatable to hamsters. In future field trials, several important rodent reservoirs of *L. major* could be targeted with ivermectin-treated baits, particularly *Rhombomys opimus* and *Meriones libycus* in parts of the Middle East and Southwest Asia, and *Arvicanthis* spp., *Mastomys* spp., and *Tatera* spp. in Sub-Saharan Africa, all of which can be baited with grains (Yaghoobi-Ershadi et al. 2000, 2005). If shown to be effective in field trials, rodent baits containing ivermectin may play a role in reducing sand fly populations, the burden of sand flies feeding on people, and the incidence of ZCL.

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